

Applicant : Yann Echelard et al.
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REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. Further the amendments merely replace the informal drawings with formal drawings. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: November 14, 2001

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“Version With Markings to Show Changes Made”

In the specification:

Paragraph beginning at page 15, line 24, has been amended as follows:

Figure 1: depicts the nucleic acid sequence of the PDGF-AB insert of expression vector pBC734 (SEQ ID NO:3). This sequence includes the nucleic acid sequence encoding human PDGF A chain, an IRES and a nucleic acid sequence encoding human PDGF B chain. This 2 kb insert was ligated into the mammary gland expression vector pBC450 (nucleic acid sequence provided; SEQ ID NO:2), to create the expression cassette pBC734. The nucleic acid sequence of the PDGF-B insert of expression vector pBC701 is also provided (SEQ ID NO:1). This insert was ligated into the mammary gland expression vector pBC450 (nucleic acid sequence provided), to create the expression cassette pBC701.

Paragraph beginning at page 36, line 10, has been amended as follows:

To create BC701, the vector pSBC-PDGF-A/-G-B was first cut partially with restriction enzyme HindIII and was ligated to the self-annealing cohesive linker HINXHO (sequence: AGCTCTCGAG; SEQ ID NO:4). Integration of this linker destroys the HindIII site and creates an Xho I site in its place. The plasmid pAB21 which had one copy of HINXHO integrated in the HindIII site located at the 3' end of the PDGF-B gene was identified using restriction enzyme mapping. Plasmid pAB21 was then partially cut with the restriction enzyme Eco RI and was ligated to the self-annealing cohesive linker ECOXHO (sequence: AATTCTCGAG; SEQ ID NO:5). Integration of this linker into an EcoRI site creates a Xho I site. The plasmid pAB23 which had one copy of ECOXHO integrated in the EcoRI site located just at the 5' end of the PDGF-B gene was identified using restriction enzyme mapping. Complete digestion of pAB23 with the restriction enzyme XhoI liberates an approximately 750 bp fragment containing the full sequence of the PDGF-B190 gene. PDGF-B190 is a specific gene construct described in detail in EP 658 198. It codes for a translation product (PDGF-BB), which is identical to fully processed mature PDGF-BB. In the construct a stop codon was introduced in position 191 of the PDGF-B precursor protein. As a

result, the carboxy-terminal part of the PDGF-B molecule, which is responsible for the retention of incompletely processed forms, is not expressed.

Paragraph beginning at page 37, line 4, has been amended as follows:

To create the PDGF-A-IRESG-PDGF-B expression cassette, the intermediate vector pAB21 was first digested to completion with the restriction enzyme NotI. The ends were filled with Klenow DNA polymerase and the resulting fragment was self-ligated. In the resulting plasmid, pAB2, the restriction site NotI located in the IRES/G sequence had been destroyed. The intermediate vector pAB2 was then cut partially with the restriction enzyme Eco RI and was ligated to the self-annealing cohesive linker ECONOXHO (sequence: AATTGCTCGAGC; SEQ ID NO:6). Integration of this linker into an EcoRI site creates and Xho I site while destroying the EcoRI site. The plasmid pAB33 which had one copy of ECONOXHO integrated in the EcoRI site located just at the 5' end of the PDGF-A gene was identified using restriction enzyme mapping. Complete digestion of pAB33 with the restriction enzyme XhoI liberates an approximately 2 kb fragment containing the full sequence of the PDGF-A gene as well as the full sequence of the PDGF-B190 gene; both genes were separated by the IRESG sequences. This 2 kb fragment was isolated and ligated into the mammary gland expression vector pBC450, to create the expression cassette pBC734 (Figure 1).

Paragraph beginning at page 38, line 31, has been amended as follows:

The following primers were used:

GBC 332: TGTGCTCCTCTCCATGCTGG(SEQ ID NO:[1] 7)

GBC 386: TGGTCTGGGTGACACATGT(SEQ ID NO:[2] 8)